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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/580,248
Filing Date: July 20, 2006
Appellant(s): ADACHI ET AL.

Robert M. Schulman
Alexander H. Spiegler
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 11/19/2009 appealing from the Office actions mailed 12/22/2008

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Tamamori-Adachi, M. "Critical Role of Cyclin D1 Nuclear Import in Cardiomyocyte Proliferation" Circ Res, vol92, (2003), pp e12-e19.

Sutterlutty, H. "P45skp2 promotes p27kip1 degradation and induces S phase in quiescent cells" Nature Cell biology, vol 1 (August, 1999), pp207-214.

Sherr, C.J. "CDK inhibitors: positive and negative regulators of G1-phase progression" Genes and Development, vol 13, (1999), pp 1501-1512.

Flink, I.L. "Changes in E2F Complexes Containing Retinoblastoma Protein Family Members and Increased Cyclin-dependent Kinase Inhibitor Activities During Terminal Differentiation of Cardiomyocytes" J Mol Cell Cardiol, vol 30, (1998), pp 563-578.

Poolman, R.A. "Altered Expression of Cell Cycle Proteins and Prolonged Duration of Cardiac Myocyte Hyperplasia in p27kip1 Knockout Mice" Circ Res, vol 85, (1999), pp 117-127.

Carrano, A.C. "SKP2 is Required for Ubiquitin-mediated Degradation of the CDK Inhibitor p27" Nature Cell Biology, vol 1, (1999), pp 193-199.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims **1, 4-12, 15-25, 31** are rejection under 35 U.S.C. 103(a) as being unpatentable over **Tamamori-Adachi et al,** [Circ Res, 92:e12-e19, 2003 (IDS)] taken with **Sutterluty et al,** (Nature Cell Biology, 1: 207-214, 1999); **Sherr et al,** [Genes & Development, 13: 1501-1512, 1999]; **Flink et al,** [J Mol Cell Cardiol, 30: 563-578, 1998 (IDS)]; and **Poolman et al,** [Circ Res, 85: 117-127, 1999 (IDS)].

Tamamori-Adachi et al teach co-expression of cyclin D1 and cyclin-dependent kinase 4 (CDK4) by using an adenovirus containing cyclin D1 gene which directly linked to nuclear localization signal (Ad-D1NLS) to target the cyclin into the nucleus and an adenovirus containing the cyclin-dependent kinase CDK4 gene (Ad-CDK4) promoted the proliferation of rat neonatal cardiomyocytes in culture (p 6, 2nd column bridge p 7, 1st column, p 7 2nd column, 2nd paragraph). Tamamori-Adachi el also teach Ad-D1NLS/ Ad-CDK4 promoted cell cycle re-entry of adult cardiomyocytes, in situ, in adult hearts injected with these viruses (p 6, 2nd column

bridge p 7, 1st column, p 7 2nd column, 2nd paragraph). Tamamori-Adachi et al suggest that postmitotic cardiomyocytes have the potential to proliferate provided that cyclin D1/CDK4 accumulate in the nucleus, and the prevention of their nuclear import plays a critical role as a physical barrier to prevent cardiomyocyte proliferation (abstract). Tamamori-Adachi et al suggest that the nucleocytoplasmic transport machinery of cyclin D1 plays a critical role for determining proliferative capacity of cardiomyocytes however, the precise mechanism preventing cyclin D1 nuclear accumulation remains unclear. Tamamori et al discuss that experiments using transgenic mice carrying wild-type cyclin D1 driven by a-cardiac myosin heavy chain (MHC) promoter have shown that deregulated wild-type cyclin D1 expression causes an increase in cardiomyocyte number and cardiomyocyte DNA synthesis in the adult heart, however, transient expression of cyclin D1 did not promote cell cycle progression in both neonatal and adult cardiomyocytes (p 7, 2nd column). Tamamori-Adachi et al suggest investigation of the molecular mechanism preventing cyclin D1 nuclear import in postmitotic cardiomyocytes will provide findings important to the development of strategies for regenerating cardiomyocytes toward the development of an alternative therapeutic application (p 8, 1st column). Tamamori-Adachi differs from the claimed invention by not teaching the introduction of a gene encoding a factor that inhibits the production or function of Cip/kip family proteins into cardiomyocyte cultures.

However, at the time of the instant invention **Sutterluty et al,** teach $p45^{skp2}$ promotes $p27^{kip1}$ degradation and induces S phase in quiescent cells (title). Sutterluty et al teach the F-box protein $p45^{skp2}$ is the substrate-targeting subunit of the ubiquitin-protein ligase SCF^{SKP2} and expression of $p45^{skp2}$ in untransformed fibroblasts activates DNA synthesis in cells that would otherwise growth-arrest and expression of $p45^{skp2}$ induces quiescent fibroblasts to enter S phase (p 207-209). Sutterluty et al, teach a vector for F-box protein $p45^{skp2}$ (p 214, under

materials and methods). Sutterluty et al, teach that expression of $p45^{skp2}$ in quiescent fibroblasts promotes $p27^{Kip1}$ degradation, allows the generation of cyclin-A-dependent kinase activity and induces S phase (abstract). Sutterluty et al propose that $p45^{skp2}$ is important in the progression from quiescence to S phase and $p45^{skp2}$ has the ability to promote $p27^{Kip1}$ degradation in cells (abstract). Sutterluty et al teach $p27^{Kip1}$ degradation, is critical for the transition from cellular quiescence to proliferation and $p27^{Kip1}$ levels are high in quiescent cells but fall once cells enter the cell cycle (p 207, 1st column, 3rd paragraph). **Sherr et al,** provides a comprehensive review of positive and negative regulators of G1-phase progression and the role of $p27^{Kip1}$ in impeding cell cycle progression and the need for $p27^{Kip1}$ degradation, in relation to cyclin D1 and CDK4 in order for the cells to progress through the cell cycle. Sherr et al, supplement the teachings of Sutterluty et al, by teaching that CDK inhibitors are positive and negative regulators of G1 to S phase transition in cells. Sherr et al teach that in the G1 phase of the cell cycle, cyclins and their corresponding kinases accumulate in the nucleus. For example cyclin D1 and CDK4 and CDK6 accumulate in the nucleus, phosphorylate retinoblastoma protein (Rb) causing its inactivation and sequester CDK inhibitors resulting in the progression of the cell cycle from G1 to S phase and CDK inhibitors such as $p27^{Kip1}$ negatively regulate cell cycle progression (p 1502, 1st column, and figure 1). Sherr et al teach that $p27^{Kip1}$ in proliferating cells is complexed to cyclin D-dependent kinases (p 1503, 1st column). In quiescent cells, the levels of $p27^{Kip1}$ are relatively high, whereas $p27^{Kip1}$ levels are low but usually increase in response to mitogenic signals during G1 phase progression (p 1503, 1st column). Titration of unbound $p27^{Kip1}$ and $p21^{Cip1}$ molecules into higher order complexes with assembling cyclin D-dependent kinases relieves cyclin E–CDK2 from Cip/Kip constraint, thereby facilitating cyclin E–CDK2 activation later in G1 phase (p 1503, 1st column). If Cip/Kip complexes cycle on and off cyclin E–CDK2, this could involve competition between

accumulating cyclin D-dependent kinases and preassembled cyclin E–CDK2 for Cip/Kip proteins (p 1503, 1st column). The levels of untethered Cip/Kip proteins may also set an inhibitory threshold for activation of cyclin E–CDK2 and cyclin A–CDK2 synthesized later in G1 phase (p 1503, 1st column). Once the process of Cip/Kip sequestration lowers the effective CKI level to a critical point, cyclin E-CDK2 can facilitate its own activation by phosphorylating $p27^{Kip1}$ on a specific threonine residue (Thr-187) to trigger its degradation. Residual $p27^{Kip1}$ and $p21^{Cip1}$ molecules remain bound to cyclin D–CDK complexes throughout subsequent cell cycles. This latent pool of tethered Cip/Kip proteins is released when mitogens are withdrawn and D cyclin synthesis stops, thereby inhibiting cyclin E–CDK2 and inducing G1 phase arrest, usually within a single cycle (p 1503, 1st column). In summary, Sherr et al teach that,, cyclin D-dependent kinases phosphorylate Rb, contributing to its inactivation;, cyclin D–CDK complexes act stoichiometrically to bind and sequester Cip/Kip proteins and the emergence of CDK2 activity during G1 requires inactivation of both the Cip/Kip proteins and Rb and is therefore dependent on prior activation of the cyclin D pathway (p 1503, 1st column last paragraph bridge to 2nd column). Therefore, Sherr et al suggest that $p27^{Kip1}$ degradation is required for cells to progress through the late G1 phase into the S phase in the comprehensive review of positive and negative regulators of G1-phase cell cycle progression. . **Flink et al,** teach that during terminal differentiation of cardiomyocytes p27 is increased (abstract). **Poolman et al,** suggests that $p27^{Kip1}$ knock out mice resulted in a significant increase in heart size and in the total number of cardiac myocytes, thus loss of $p27^{Kip1}$ results in prolonged proliferation of the mouse cardiac myocytes (abstract). The combination of Sutterluty, Sherr, Flink and Poolman suggest the requirement of $p27^{Kip1}$ degradation in order for the cells to progress from the G1 to S phase of the cell cycle and the role of $p27^{Kip1}$ in terminal differentiation of cardiomyocytes while its loss is associated with cardiomyocyte cell proliferation. As such the combination of Sutterluty, Sherr,

Flink, and Poolman provide sufficient motivation for one of ordinary skill in the art to introduce a gene encoding a factor that inhibits the production or function of $p27^{kip1}$ to the cardiomyocyte system of Tamamori-Adachi in order to promote the progression of terminally differentiated cardiomyocytes through the G1 to S phase.

Accordingly, in view of the combination of the teachings of Sutterluty, Sherr, Flink, and Poolman, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to co-transfect with the Ad-D1NLS/ Ad-CDK4 the vector for F-box protein $p45^{skp2}$ to the cardiomyocyte cell culture system of Tamamori-Adachi in order to inhibit the production of the $p27^{kip1}$ gene in cultured cardiomyocytes with a reasonable expectation of success. One of ordinary of skill in the art would have been sufficiently motivated to introduce into the cardiomyocytes of Tamamori-Adachi which are coinfected with Ad-D1NLS/ Ad-CDK4 the $p45^{skp2}$ vector of Sutterluty since Sutterluty teaches that $p45^{skp2}$ is important for the progression of cells from the quiescence to S phase and because $p45^{skp2}$ has the ability to promote $p27^{Kip1}$ degradation and particularly since Sherr et al suggest that $p27^{Kip1}$ degradation is required for cells to progress through the late G1 phase into the S phase and in addition, since Fink teaches p27 is increased during cardiomyocyte differentiation. One of ordinary skill in the art would have been sufficiently motivated to co-introduce the $p27^{kip1}$ inhibitor in terminally differentiated cardiomyocytes as Flink et al, teach that during terminal differentiation of cardiomyocytes p27 is increased taken with Poolman's suggestions that $p27^{Kip1}$ knock out mice resulted in a significant increase in heart size and in the total number of cardiac myocytes, thus loss of $p27^{Kip1}$ results in prolonged proliferation of the mouse cardiac myocytes. One of ordinary skill in the art would have been sufficiently motivated to make such a modification because Tamamori-Adachi et al teach that the nucleocytoplasmic transport of cyclin D1 plays a critical role for determining proliferative capacity of cardiomyocytes and suggest investigation of the

molecular mechanism preventing cyclin D1 nuclear import in postmitotic cardiomyocytes will provide findings important to the development of strategies for regenerating cardiomyocytes toward the development of an alternative therapeutic application.

Thus, the claimed invention as a whole is clearly prima facie obvious in the absence of evidence to the contrary.

Claims **34-35** are rejected under 35 U.S.C. 103(a) as being unpatentable over **Tamamori-Adachi et al,** [Circ Res, 92:e12-e19, 2003 (IDS)] taken with **Sutterluty et al,** (Nature Cell Biology, 1: 207-214, 1999); **Sherr et al,** [Genes & Development, 13: 1501-1512, 1999, (IDS)]; **Flink et al,** [J Mol Cell Cardiol, 30: 563-578, 1998 (IDS)]; and **Poolman et al,** [Circ Res, 85: 117-127, 1999 (IDS)] and further in view of **Carrano et al,** [Nature Cell Biology, 1: 193-199, (IDS)].

The teachings of Tamamori-Adachi et al, taken with Sutterluty et al, taken with Sherr et al, taken with Flink et al, taken with Poolman et al, are applied here as mentioned above.

The above combined references teach the importance of co-transfection of adult or neonatal cardiomyocytes with the Ad-D1NLS/Ad-CDK4 and the p45skp2 in order to induce cell cycle progression thus increased cell proliferation compared to control cardiomyocytes. The teachings of Carrano et al supplement the teachings of the combined references of Tamamori-Adachi/Sutterlutty/Sherr/Flink and Poolman which teachi that degradation of the mammalian cyclin-dependent kinase (CDK) inhibitor $p27^{kip1}$ is required for the cellular transition from quiescence to the proliferative state by providing the p45skp2 vector and emphasizes that p45skp2 is required for ubiquitin-mediated degradation of the $p27^{Kip1}$ for cellular transition from quiescence to the proliferative state in HeLa cells (abstract, and p 196, figure 4)). Carrano teaches that $p27^{Kip1}$ degradation is accomplished via SKP2 ubiquitination. Thus, Carrano

supplements the teachings of Sutterluty by teaching the requirement for SKP2 in $p27^{Kip1}$ ubiquitination, together with the need for cyclin E-CDK2 which indicates the existence of a dual control mechanism for $p27^{Kip1}$ degradation during cell cycle progression (p 198, 2nd column, 2nd paragraph). Moreover, Carrano suggests the $p27^{kip1}$ ubiquitination occurs during the S-phase (proliferation phase), when $p27^{kip1}$ levels are kept low by the high levels of SKP2 (p 198, 2nd column, 2nd paragraph). Therefore, the combination of the cited references clearly suggest, that co-transfection of cardiomyocytes with the Ad-D1NLS/Ad-CDK4 and the p45skp2 to cardiomyocytes would induce cell cycle progression at different phases of the cell cycle including the GO phase of the cell cycle when the cells are withdrawn from the cell cycle compared to control cardiomyocytes. Therefore, the adult cardiomyocytes as taught by the combined cited references provides the method for the proliferating cardiomyocytes that have withdrawn from the cell cycle comprised of Ad-D1NLS/Ad-CDK4 and the p45skp2 in order to induce cell cycle progression.

Tamamori-Adachi/Sutterluty//Sherr/Flink/Poolman/Carrano, taken together, provide teaching, suggestion, and motivation to perform the instantly claimed methods.

The instant claims combine the elements co-transfection of cardiomyocytes with the Ad-D1NLS/Ad-CDK4 and the p45skp2 to cardiomyocytes would induce cell cycle progression at different phases of the cell cycle including the GO phase of the cell cycle when the cells are withdrawn from the cell cycle compared to control cardiomyocytes. . Supreme Court reaffirmed principles based on its precedent

that “[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.” KSR International Co. v. Teleflex Inc. (KSR), 550 U.S. at, 82 USPQ2d at 1395.

Therefore, in view of Tamamori-Adachi/Sutterluty//Sherr/Flink/Poolman/Carrano it would be

prima facie obvious for one of skill in the art to transfect adult cardiomyocytes with the Ad-D1NLS/Ad-CDK4 and the p45skp2 to cardiomyocytes would induce cell cycle progression at different phases of the cell cycle including the GO phase of the cell cycle when the cells are withdrawn from the cell cycle compared to control cardiomyocytes

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

(10) Response to Argument

Appellant's arguments have been addressed in the order in which they have been presented in the appellant's appeal brief.

1) Whether the rejection of Claims **1-2, 4-12, 15-25, 31** under 35 U.S.C. 103(a) as being unpatentable over **Tamamori-Adachi et al,** [Circ Res, 92:e12-e19, 2003 (IDS)] taken with **Sutterluty et al,** (Nature Cell Biology, 1: 207-214, 1999); **Sherr et al,** [Genes & Development, 13: 1501-1512, 1999, (IDS)]; **Flink et al,** [J Mol Cell Cardiol, 30: 563-578, 1998 (IDS)]; and **Poolman et al,** [Circ Res, 85: 117-127, 1999 (IDS)] is proper.

A. The Appellant argues on pages 3-5 of the appeal brief, that the USPTO alleges that Adachi teaches claim 1, elements (a) and (b), but does not teach "the introduction of a gene encoding a factor that inhibits the production or function of Cip/Kip family proteins into cardiomyocyte cultures." The USPTO contends, however, that it would have been obvious to introduce such a gene into Adachi's system in view of Sutterluty Sherr, Flink, and Poolman. Appellants argue first, the cited references alone, or in combination, do not teach or suggest each and every claim element. Indeed, Sutterluty, Sherr, Flink, and Poolman alone, or in combination, do not teach or suggest claim 1, element (c), i.e., introducing a gene encoding a factor that inhibits the production, function, or action of Cip/Kip family protein into

cardiomyocytes in vitro. Second, even if these references did teach element (c), which they do not, there is no reason to combine this hypothetical teaching with Adachi. Indeed, the evidence of record demonstrates that the introduction of a gene encoding a factor that inhibits the production, function, or action of Cip/Kip protein does riot result in the proliferation of cardiomyocytes. As such, one of ordinary skill in the art would have had no reason to combine the hypothetical teaching of element (c) with Adachi to arrive at the claimed invention.

These arguments are not persuasive because Talmadori-Adachi et al teach co-expression of cyclin D1 and cyclin-dependent kinase 4 (CDK4) by using an adenovirus containing the cyclin D1 gene which directly linked to nuclear localization signal (Ad-D1NLS) to target the cyclin into the nucleus and an adenovirus containing the CDK4 gene (Ad-CDK4) promoted the proliferation of rat neonatal cardiomyocytes in culture (p 6, 2nd column bridge p 7, 1st column, p 7 2nd column, 2nd paragraph). Second, Tamamori-Adachi el also teach Ad-D1NLS/ Ad-CDK4 promoted cell cycle re-entry of adult cardiomyocytes, in situ, in adult hearts injected with these viruses (p 6, 2nd column bridge p 7, 1st column, p 7 2nd column, 2nd paragraph). Thus, Tamamori-Adachi explicitly teaches the nuclear import of cyclin D1/CDK4 promotes cell cycle entry of adult cardiomyocytes in vivo (see page 7, 1st column, 1st paragraph). Clearly, Tamari-Adachi teaches elements (a) and (b) as instantly claimed. Third, obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In the instant case, one of ordinary of skill in the art would have been sufficiently motivated to introduce into the cardiomyocytes of Tamamori-Adachi which are coinfected with Ad-D1NLS/ Ad-CDK4 the $p45^{skp2}$ vector of

Sutterluty since Sutterluty teaches that $p45^{skp2}$ is important for the progression of cells from the quiescence to S phase and because $p45^{skp2}$ has the ability to promote $p27^{Kip1}$ degradation and particularly since Sherr et al suggest that $p27^{Kip1}$ degradation is required for cells to progress through the late G1 phase into the S phase. In addition, since Fink teaches p27 is increased during cardiomyocyte differentiation. One of ordinary skill in the art would have been sufficiently motivated to co-introduce the $p27^{kip1}$ inhibitor in terminally differentiated cardiomyocytes as Flink et al, teach that during terminal differentiation of cardiomyocytes p27 is increased taken with Poolman's suggestions that $p27^{Kip1}$ knock out mice resulted in a significant increase in heart size and in the total number of cardiac myocytes, thus loss of $p27^{Kip1}$ results in prolonged proliferation of the mouse cardiac myocytes. Furthermore, since Sutterlutty already taught that p27kip is degraded via the ubiquitination molecule p45skp2 it is obvious for a skill in the art of cell cycle regulation to introduce said molecule to degrade p27kip1 in order to progress the cardiomyocytes through the cells cycle thus inducing proliferation of the cardiomyocytes because al these molecules are required to promote cell cycle progression as taught by the cited references as a whole. The combination of the cited references clearly suggest, that would exit the cells from the quiescent phase of the cell cycle and s would induce cell cycle progression thus increase cell proliferation of quiescent cardiomyocytes.

Appellants argue that the evidence of record demonstrates unexpected results. Appellants argue that the introduction of a gene encoding a factor that inhibits the production, function, or action of Cip/Kip protein, without elements (a) and (b), does not result in the proliferation of cardiomyocytes. Thus, one of ordinary skill in the art would have expected no increase in proliferation if this gene (e.g., Skp2) was introduced into cardiomyocytes and co-expressed with other genes as compared to the co-expression of these other genes alone. The specification teaches, however, that the co-expression of Skp2, cyclin D, and CDK4 resulted in

a significant increase in cell number as compared to the co-expression of only cyclin D and CDK4. Accordingly, Appellants respectfully submit that the evidence of unexpected results is sufficient to overcome a prima facie case of obviousness.

These arguments are not persuasive because in contrast to Appellant's assertion there is no evidence of unexpected results on record. Appellants have not provided evidence that following co-expression of Skp2, cyclin D, and CDK4 would not result in increased cell proliferation as compared to expression of these genes alone in cardiomyocytes as set forth in the instant rejection in view of the foregoing combination of the cited references. In contrast to the Appellant's assertion, the instant invention is directed to the co-expression of elements (a) and (b) and (c) and not element (c) alone for increased proliferation of cardiomyocytes. As set forth in the instant rejection the co-expression of elements (a) (b) and (c) promotes cell cycle progression of cardiomyocytes from the G0/G1 into the S-phase, therefore, resulting in increased cell proliferation. Moreover, Carrano asserts the p45skp2 is important in the control of cell proliferation via cellular transition from quiescence into proliferative state that is the S-phase where cells are highly proliferative (emphasis added).

B. **1.** Appellants argue the USPTO has the burden of establishing a prima facie case of obviousness. An "expansive and flexible approach" should be applied when determining obviousness based on a combination of prior art references. However, a claimed invention combining multiple known elements is not rendered obvious simply because each element was known independently in the prior art. Rather, there must still be some "reason that would have prompted" a person of ordinary skill in the art to combine the elements in the specific way that he or she did. Also, modification of a prior art reference may be obvious only if there exists a reason that would have prompted a person of ordinary skill to make the change: Accordingly,

an obviousness rejection "cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness."

These arguments are not persuasive because in the instant case, one of ordinary of skill in the art would have been sufficiently motivated to introduce into the cardiomyocytes of Tamamori-Adachi which are coinfected with Ad-D1NLS/ Ad-CDK4 the $p45^{skp2}$ vector of Sutterluty since Sutterluty teaches that $p45^{skp2}$ is important for the progression of cells from the quiescence to S phase and because $p45^{skp2}$ has the ability to promote $p27^{Kip1}$ degradation and particularly since Sherr et al suggest that $p27^{Kip1}$ degradation is required for cells to progress through the late G1 phase into the S phase. In addition, since Fink teaches p27 is increased during cardiomyocyte differentiation. One of ordinary skill in the art would have been sufficiently motivated to co-introduce the $p27^{kip1}$ inhibitor in terminally differentiated cardiomyocytes as Flink et al, teach that during terminal differentiation of cardiomyocytes p27 is increased taken with Poolman's suggestions that $p27^{Kip1}$ knock out mice resulted in a significant increase in heart size and in the total number of cardiac myocytes, thus loss of $p27^{Kip1}$ results in prolonged proliferation of the mouse cardiac myocytes. Furthermore, since Sutterlutty already taught that p27kip is degraded via the ubiquitination molecule p45skp2 it is obvious one of ordinary skill in the art of cell cycle regulation to introduce said molecule to degrade p27kip1 in order to progress the cardiomyocytes through the cells cycle thus inducing proliferation of the cardiomyocytes because al these molecules are required to promote cell cycle progression as taught by the cited references as a whole. The combination of the cited references clearly suggest, that would exit the cells from the quiescent phase of the cell cycle and would induce cell cycle progression thus increase cell proliferation of quiescent cardiomyocytes.

2. **a.** Appellants argue that Adachi does not teach or suggest the involvement of the Cip/Kip family protein (e.g., p27Kipl) in the proliferation process of cardiomyocytes. Rather, as discussed in the specification, Adachi discloses a novel method of proliferating cardiomyocytes comprising the expression of a cyclin and a CDK. Accordingly, Adachi does not teach or suggest claim element 1 (c).

These arguments are not persuasive because while Adachi does not teach element (c) however, Sherr clearly teaches the mechanism as to why $p27^{kip1}$ degradation is required fro cells to progress though the cell cycle from late G1 phase into the S phase relatively to cyclin D1, cyclin D1-dependent kinase phopshorylation of Rb and both Cip/kip proteins. Sherr suggests that $p27^{kip1}$degradation is required for cells to progress through the late G1 phase to S phase. Flink by teaching that in differentiated cardiomyocytes p27 is increased, this is sufficient motivation for one of skill in the art of cell cycle regulation to degrade p27 in the cardiomyocytes of Talmadori-Adachi, particularly by the teachings of Poolman where total loss of p27 in the knock out mice resulted in prolonged proliferation of cardiomyocytes (emphasis added). Furthermore, since Sutterlutty already taught that $p27^{kip1}$ is degraded via the ubiquitination molecule $p45^{skp2}$ it is obvious for a skill in the art of cell cycle regulation to introduce said molecule to degrade $p27^{kip1}$ in order to progress the cardiomyocytes through the cells cycle thus inducing proliferation of the cardiomyocytes. Therefore, the combination of the cited references clearly suggest, that co-transfection of cardiomyocytes with the Ad-D1NLS/Ad-CDK4 and the $p45^{skp2}$ to cardiomyocytes would induce cell cycle progression thus increased cell proliferation compared to control cardiomyocytes.

b. Appellants argue that Sutterluty does not teach or relate to methods of proliferating cardiomyocytes. Sutterluty relates to cell-cycle mechanisms in fibroblasts--actively dividing cells physiologically distinct from cardiomyocytes. Sutterluty does not discuss or provide any teaching

whatsoever with respect to cardiomyocytes or methods of proliferating cardiomyocytes. Rather, Sutterluty directs one of ordinary skill in the art to consider its teachings with the development of colorectal and breast cancers. Accordingly, Sutterluty is unrelated to the claimed invention.

These arguments are not persuasive because Sutterluty is not cited for the cell type but specifically is cited for the Ad.SKP2 vector ($p45^{skp2}$) and its ability to promote $p27^{kip1}$ degradation and induces S phase in quiescent cells (title). Sutterluty teaches that $p45^{skp2}$ is important for the progression of cells from the quiescence to S phase (abstract). Furthermore, since Sutterlutty already taught that $p27^{kip1}$ is degraded via the ubiquitination molecule ($p45^{skp2}$) it is obvious for one of ordinary skill in the art of cardiomyocyte cell cycle regulation to introduce the Ad.SKP2 vector ($p45^{skp2}$) to degrade $p27^{kip1}$ in order to progress the cardiomyocytes through the cells cycle thus inducing proliferation of the cardiomyocytes. Therefore, the combination of the cited references clearly suggest, that co-transfection of cardiomyocytes with the Ad-D1NLS/Ad-CDK4 and the $p45^{skp2}$ and the association of these molecules with cell cycle progression and inducing cardiomyocyte proliferation by degradation of $p27^{kip1}$ via the ubiquitination molecule ($p45^{skp2}$).

c. Appellants argue that Sherr does not relate to cardiomyocytes and does not teach or suggest introducing a gene encoding a factor to inhibit production or function of a Cip/Kip protein. Sherr, like Sutterluty, is silent with respect to cardiomyocytes and methods of proliferating cardiomyocytes.

These arguments are not persuasive because Sherr is cited to emphasize the mechanism as to how during the cell cycle progression $p27^{Kip1}$ degradation is required for cells to progress through the late G1 phase into the S phase. Therefore, Sherr supplements the teachings of Sutterluty et al, by teaching that $p27^{Kip1}$ in proliferating cells is complexed to cyclin D-dependent kinases (p 1503, 1st column) and in quiescent cells, the levels of $p27^{Kip1}$ are

relatively high, whereas $p27^{Kip1}$ levels are low but usually increase in response to mitogenic signals during G1 phase progression (p 1503, 1st column).

d. Appellants argue that Flink does not teach or suggest the inhibition of Cip/Kip proteins, let alone teach or suggest modes of inhibiting such proteins. According to the USPTO, "Flink by teaching that in differentiated cardiomyocytes p27 is increased, this is sufficient motivation for one of skill in the art of cell cycle regulation to degrade p27 in the cardiomyocytes of [Adachi]." Flink provides no such motivation. Flink is a general reference reporting the changes in E2F complexes containing retinoblastoma protein family members and cyclin-dependent kinase inhibitor activities during terminal differentiation of cardiomyocytes. At best, Flink suggests that a paradox exists for p27, where mRNA levels are stable, but protein levels increase. Flink does not discuss or provide any teachings whatsoever regarding the inhibition of Cip/Kip proteins, let alone teach or suggest modes of inhibiting such proteins. Flink is also silent on methods of proliferating cardiomyocytes. Adachi discloses a method of proliferating cardiomyocytes by introducing a cyclin and cyclin-dependent kinase, but is silent with respect to p27. The USPTO has not established a nexus between Flink and Adachi. Indeed, the USPTO does not provide any reasoning why one of ordinary skill in the art reading Flink would have looked to Adachi. Accordingly, the USPTO fails to support its assertion that Flink "provides sufficient motivation ... to degrade p27 in the cardiomyocytes of [Adachi]."

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In the instant case, Flink is cited for teachings that during terminal differentiation of cardiomyocytes $p27^{Kip1}$ is increased (abstract). Flink links the role of $p27^{Kip1}$ into the cardiomyocytes. Flink teaches the role of

$p27^{Kip1}$ in cardiomyocytes and Sutterlutty teaches the role of $p45^{skp2}$ is important in the progression from quiescence to S phase and $p45^{skp2}$ has the ability to promote $p27^{Kip1}$ degradation in quiescent fibroblasts. Therefore, Flink and Sutterlutty together teach the need of these molecules for the progression of cells through the cell cycle thus for the progression of quiescent cardiomyocytes into cell cycle and cardiomyocyte proliferation. Regarding the Tamamori-Adachi reference is cited for the need of Ad-D1NLS/ Ad-CDK4 molecules in order for promote cell cycle re-entry of adult cardiomyocytes. Therefore, it is the combination of the above molecules as taught by the combination of the cited references in order to promote cell cycle progression of quiescent cardiomyocytes.

e. Appellants argue that Poolman does not teach or suggest that introducing a gene encoding a factor that inhibits the production, function or action of Cip/Kip protein. Poolman is limited to the developmental effects of the absence (i.e., the total loss) of p27 in neonatal cardiomyocytes. In particular, Poolman suggests that a genetically engineered mouse lacking $p27^{Kip1}$ (i.e., $p27^{Kip1}$ knockout mouse) showed "prolonged proliferation of cardiac myocytes." Poolman does not teach or suggest inhibiting the production or function of a Cip/Kip protein, nor introducing a gene encoding a factor to accomplish such inhibition. Indeed, one of ordinary skill in the art would appreciate that the developmental events influenced by the complete absence of $p27^{Kip1}$ during development are not identical to inhibiting a Cip/Kip protein. Furthermore, the specification provides evidence that almost no increase in the cell number of cardiomyocytes was observed where the production of $p27^{Kip1}$ gene product was inhibited by infection with p27 siRNA alone. The USPTO acknowledges that Poolman teaches the total loss of p27, but fails to address the distinction between a knockout mouse and the introduction of a gene encoding a factor to inhibit production or function of a Cip or Kip protein. In view of the foregoing, the combination of Sutterlutty, Sherr, Flink, and Poolman does not teach or suggest a method of

introducing a gene encoding a factor that inhibits production or function of Cip/Kip protein into cardiomyocytes in vitro. Sutterlutty and Sherr do not relate at all to cardiomyocytes or methods of proliferating cardiomyocytes. Rather, these references direct one of ordinary skill in the art to consider cell cycle mechanisms in various cancers. Flink relates to cardiomyocytes, but is silent with respect to proliferating cardiomyocytes or inhibiting Cip/Kip proteins. Poolman discloses a p27 knockout mouse, but does not teach or suggest introducing an exogenous factor into cardiomyocytes. Accordingly, the combination of references does not teach or suggest claim 1, element (c).

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In the instant case, Flink is cited for teachings that during terminal differentiation of cardiomyocytes $p27^{Kip1}$ is increased (abstract). Flink links the role of $p27^{Kip1}$ into the cardiomyocytes. Flink teaches the role of $p27^{Kip1}$ in cardiomyocytes and Sutterlutty teaches the role of $p45^{skp2}$ is important in the progression from quiescence to S phase and $p45^{skp2}$ has the ability to promote $p27^{Kip1}$ degradation in quiescent fibroblasts. Therefore, Flink and Sutterlutty together teach the need of these molecules for the progression of cells through the cell cycle thus for the progression of quiescent cardiomyocytes into cell cycle and cardiomyocyte proliferation. Regarding the Tamamori-Adachi reference is cited for the need of Ad-D1NLS/ Ad-CDK4 molecules in order for promote cell cycle re-entry of adult cardiomyocytes. Poolman et al, suggests that $p27^{Kip1}$ knock out mice resulted in a significant increase in heart size and in the total number of cardiac myocytes, thus loss of $p27^{Kip1}$ results in prolonged proliferation of the mouse cardiac myocytes (abstract). . Therefore, the combination of Sutterluty, Sherr, Flink and Poolman suggest the

requirement for $p27^{Kip1}$ degradation in order for the cells to progress from the G1 to S phase of the cell cycle and the role of $p27^{Kip1}$ in terminal differentiation of cardiomyocytes is to impede progression to the S phase, while its loss is associated with cell cycle progression thus, inducing cardiomyocyte cell proliferation as set forth in the instant rejection of the combined references in the adult cardiomyocyte system. As such the combination of Sutterluty, Sherr, Flink, and Poolman provide sufficient motivation for one of ordinary skill in the art to introduce a gene encoding a factor that inhibits the production or function of $p27^{kip1}$ to the cardiomyocyte system of Tamamori-Adachi in order to promote the progression of terminally differentiated cardiomyocytes through the G1 to S phase.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., the specification provides evidence that almost no increase in the cell number of cardiomyocytes was observed where the production of $p27^{Kip1}$ gene product was inhibited by infection with p27 siRNA alone) is not related to the instant invention. . Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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